

The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

1-23. (Cancelled)

24. (Withdrawn) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

25. (Withdrawn) The method of claim 24, wherein said nucleic acid template is a DNA molecule.

26. (Withdrawn) The method of claim 25, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

27. (Withdrawn) The method of claim 26, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UItma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

28-30. (Cancelled)

31. (Withdrawn) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

32. (Withdrawn) A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

33. (Withdrawn) The method of claim [29,]31, or 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

34. (Withdrawn) The method of claim 24 [33], wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

35. (Withdrawn) The method of claim 24, 31 or 32, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

36. (Withdrawn) An isolated mutant DNA polymerase comprising a reduced DNA polymerization activity.

37. (Withdrawn) An isolated mutant DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

38. (Withdrawn) The mutant DNA polymerase of claim 36 or 37, wherein said mutant DNA polymerase comprises a mutation in the partitioning domain or in the polymerase domain.

39. (Withdrawn) The mutant DNA polymerase of claim 37, wherein said mutant DNA polymerase is a mutant Pfu DNA polymerase.

40. (Withdrawn) A mutant Pfu DNA polymerase with reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

41. (Withdrawn) The mutant DNA polymerase of claim 40, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

42. (Withdrawn) The mutant DNA polymerase of claim 37, wherein said mutant DNA polymerase is derived from the group consisting of: Ultma DNA polymerase, Tli DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

43. (Withdrawn) A composition for DNA synthesis comprising an isolated mutant DNA polymerase which comprises a reduced DNA polymerization activity.

44. (Withdrawn) A composition for DNA synthesis comprising an isolated mutant DNA polymerase which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity.
45. (Withdrawn) The composition of claim 43 or 44, wherein said mutant DNA polymerase comprises a mutation in the partitioning domain or the polymerase domain.
46. (Withdrawn) The composition of claim 44, wherein said mutant DNA polymerase is a mutant Pfu DNA polymerase.
47. (Withdrawn) A composition comprising a mutant Pfu DNA polymerase, wherein said mutant DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.
48. (Withdrawn) The composition of claim 47, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
49. (Withdrawn) The composition of claim 44, wherein said mutant DNA polymerase is derived from the group consisting of: U1Tma DNA polymerase, Tli DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
50. (Withdrawn) A kit for DNA synthesis comprising a mutant DNA polymerase which comprises a reduced DNA polymerization activity and packaging material therefore.
51. (Withdrawn) A kit for DNA synthesis comprising a mutant DNA polymerase which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity and packaging material therefore.
52. (Withdrawn) The kit of claim 50 or 51, wherein said mutant DNA polymerase comprises a mutation in the partitioning domain or the polymerase domain.
53. (Withdrawn) The kit of claim 51, wherein said mutant DNA polymerase is a mutant Pfu DNA polymerase.
54. (Withdrawn) A kit comprising a mutant DNA polymerase which comprises a reduced DNA polymerization activity and packaging material therefor, wherein said mutant Pfu DNA

polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

55. (Withdrawn) The kit of claim 54, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

56. (Withdrawn) The kit of claim 51, wherein said mutant DNA polymerase is derived from the group consisting of: U1Tma DNA polymerase, Tli DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

57. (Withdrawn) A mutant Pfu DNA polymerase produced by introducing a mutation in to a polynucleotide encoding a wild type Pfu DNA polymerase to produce a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

58. (Withdrawn) A mutant Pfu DNA polymerase comprising a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase is produced by the steps:

(a) providing a polynucleotide encoding a wild-type Pfu DNA polymerase;

(b) introducing one or more nucleotide mutations into said polynucleotide to produce a mutant polynucleotide encoding said mutant Pfu DNA polymerase; and

(c) expressing said mutant polynucleotide to produce said mutant Pfu DNA polymerase, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

59. (Withdrawn) The mutant DNA polymerase of claim 58, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

60. (Withdrawn) A composition comprising a mutant Pfu DNA polymerase produced by expressing a polynucleotide encoding a Pfu DNA polymerase with a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

61. (Withdrawn) A composition comprising a mutant Pfu DNA polymerase comprising a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase is produced by the steps:

(a) introducing a mutation into a polynucleotide encoding a wild-type Pfu DNA polymerase to produce said mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388;

(c) expressing said mutant polynucleotide to produce said composition comprising said mutant Pfu DNA polymerase.

62. (Withdrawn) The composition of claim 60 or 61, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

63. (Withdrawn) The method of claim 33, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

64. (Previously presented) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is an Archaeal DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein the mutant Archaeal DNA polymerase comprises a mutation selected from the group consisting of amino acid positions corresponding to D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388 of Pfu DNA polymerase.

65. (Currently Amended) The enzyme mixture of claim 64, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: Tli DNA polymerase (Vent DNA polymerase), PGB-D (Deep Vent) DNA polymerase, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, and JDF-3 DNA polymerase (SEQ ID NO. 10).

66. (Previously presented) The enzyme mixture of claim 65, wherein said mutant DNA polymerase comprises a mutation in its partitioning domain or polymerase domain.

67. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA

~~polymerization activity, wherein said mutant DNA polymerase comprises a mutation at a position as indicated in Tables 2A and 2B, wherein when said mutant Archaeal DNA polymerase is a mutant Pfu DNA polymerase, and said mutant Pfu DNA polymerase contains a mutation at an amino acid position selected from the group consisting of Y410, T542, D543, K593, Y595, Y385, G387, and G388.~~

68. (Currently Amended) The enzyme mixture of claim 67, wherein said mutant DNA polymerase comprising a mutation in its partitioning domain or polymerase domain is a mutant Pfu DNA polymerase, KOD DNA polymerase, Tgo DNA polymerase, Tli (Vent) DNA polymerase, PGB-D (Deep Vent), or JDF-3 DNA polymerase (SEQ ID NO. 10).

69. (Cancelled)

70. (Currently Amended) The enzyme mixture of claim 679, wherein said mutant Pfu DNA polymerase contains a mutation of G387P.

71. (Currently Amended) The enzyme mixture of claim 68, wherein said mutant DNA polymerase is a mutant KOD DNA polymerase, and said mutant KOD DNA polymerase contains a mutation at an amino acid position selected from the group consisting of Y384, G386, G387, D404, T541, D542, and K592.

72. (Currently Amended) The enzyme mixture of claim 71, wherein said mutant KDO KOD DNA polymerase contains a mutation of G387P.

73. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a ~~The enzyme mixture of claim 68, wherein said mutant JDF-3 DNA polymerase(SEQ ID NO. 10) contains a mutation at amino acid position G387.~~

74. (Currently Amended) The enzyme mixture of claim 73, wherein said mutant JDF-3 DNA polymerase contains a mutation of G387P.

75. (Previously presented) The enzyme mixture of claim 64, wherein said first enzyme and said second enzyme are derived from two different Archaeal DNA polymerases.

76. (Currently Amended) The enzyme mixture of claim 75, wherein said first enzyme is wild type KOD or wild type JDF-3 DNA polymerase (SEQ ID NO. 10), and said second enzyme is a mutant Pfu DNA polymerase.

77. (Previously presented) The enzyme mixture of claim 76, wherein said mutant Pfu DNA polymerase contains a mutation at amino acid G387.

78. (Previously presented) The enzyme mixture of claim 77, wherein said mutant Pfu DNA polymerase contains a mutation of G387P.

79. (Previously presented) The enzyme mixture of claim 75, wherein said first enzyme is wild type Pfu DNA polymerase, and said second enzyme is a mutant KOD or mutant JDF-3 DNA polymerase.

80. (Previously presented) The enzyme mixture of claim 79, wherein said mutant KOD or mutant JDF-3 DNA polymerase contains a mutation of G387.

81. (Previously presented) The enzyme of claim 80, wherein said mutant KOD or mutant JDF-3 DNA polymerase contains a mutation of G387P.

82. (Previously presented) The enzyme mixture of claim 67, wherein said first enzyme is Taq DNA polymerase.

83. (Previously presented) The enzyme mixture of claim 82, wherein said second DNA polymerase is a mutant Pfu, a mutant KOD or a mutant JDF-3 DNA polymerase.

84. (Previously presented) The enzyme of claim 83, wherein said mutant Pfu, mutant KOD, or mutant JDF-3 DNA polymerase contains a mutation of G387P.

85. (Previously presented) A kit comprising an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is an Archaeal DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein the mutant Archaeal DNA polymerase comprises a mutation selected from the group consisting of amino acid positions corresponding to D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388 of Pfu DNA polymerase, and packaging material therefor.

86. (Currently Amended) A kit comprising an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein said mutant DNA polymerase comprises a mutation at a position as indicated in Tables 2A and 2B, and packaging material therefor, wherein when said mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity is a mutant Pfu DNA polymerase, and said mutant Pfu DNA polymerase contains a mutation at an amino acid position selected from the group consisting of Y410, T542, D543, K593, Y595, Y385, G387, and G388.

87. (Previously presented) The kit of claim 85 or 86, further comprising a reagent selected from the group consisting of: dNTPs, reaction buffer, primer, and DNA enhancing factor.

88. (New) The enzyme mixture of claim 74, wherein said mutant JDF-3 DNA polymerase contains a mutation of G387P.

89. (New) The enzyme mixture of claim 68, wherein when said mutant DNA polymerase comprising a mutation in its partitioning domain or polymerase domain is a mutant Tgo DNA polymerase, said mutant Tgo DNA polymerase contains a mutation at an amino acid position selected from the group consisting of: D404, T541, D542, K592, Y384, G386, and G387.

90. (New) The enzyme mixture of claim 89, wherein said mutant Tgo DNA polymerase contains a mutation of G386P.

91. (New) The enzyme mixture of claim 68, wherein when said mutant DNA polymerase comprising a mutation in its partitioning domain or polymerase domain is a mutant Tli (Vent) DNA polymerase, said mutant Tli (Vent) DNA polymerase contains a mutation at an amino acid position selected from the group consisting of: D407, T544, D545, K595, Y387, G389, and G390.

92. (New) The enzyme mixture of claim 91, wherein said mutant Tli (Vent) DNA polymerase contains a mutation of G389P.

93. (New) The enzyme mixture of claim 68, wherein when said mutant DNA polymerase comprising a mutation in its partitioning domain or polymerase domain is a mutant PGB-D (Deep Vent) DNA polymerase, said mutant PGB-D (Deep Vent) DNA polymerase contains a mutation at an amino acid position selected from the group consisting of: D405, T542, D543, K593, Y385, G387, and G388.

94. (New) The enzyme mixture of claim 93, wherein said mutant PGB-D (Deep Vent) DNA polymerase contains a mutation of G387P.